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Resilience of benthic diatom communities in Mediterranean streams: role of endangered species

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Abstract. The extent of drought in Mediterranean streams has been intensifying recently, and the mean annual discharge is expected to experience a decreasing trend in coming years, with significant effects on aquatic ecosystems. The aim of this study was to analyse colonisation patterns of diatom communities that differed in terms of taxonomic composition and percentage of endangered taxa exploring the possible development of resistance mechanisms. To this end, we selected three Mediterranean streams comparable in terms of water quality, but different in terms of surrounding land use, and we performed two experimental treatments. The first treatment consisted in artificially drying and cleaning of substrates (cobbles) to analyse the post-drought recolonisation process that is only driven by drift and immigration. In the second treatment cobbles coming from a site experiencing a seasonal drought were transplanted upstream in a perennial stretch to explore the possible development of resistance mechanisms within diatom communities periodically exposed to droughts. We observed that stream identity played an important role in determining diatom assemblage composition. Highly natural stretches had a high abundance of endangered species, which were less resilient to drought than assemblages composed of general and widespread taxa. Moreover, according to our results, resistance mechanisms did not play a significant role in recovery patterns. Improving our knowledge of diatom resilience mechanisms is very important in a global climate change scenario, especially in Mediterranean streams.

Additional keywords: Bacillariophyceae, colonisation patterns, hydrological intermittency, Red List species.

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Introduction

Seasonal droughts in Mediterranean intermittent streams represent a natural part of their hydrological cycle; the survival of benthic organisms inhabiting these ecosystems is mainly related to the evolution of resistance and resilience strategies.

Resistance is the ability of organisms to exploit potential refuges during dry periods, or their capacity to produce resistant forms. For example, isolated pools, which form during the final phases of the lentification process, can be considered important refuges for aquatic biota (Robson and Matthews 2004; Falasco *et al.* 2016). Moreover, when sufficient humidity is retained, biofilm on cobbles is also an important shelter for benthic primary producers (Chester and Robson 2014; Sabater *et al.* 2016), because the extracellular polymeric substances (EPS) represent a layer for protection against desiccation (Gorbushina 2007; Sabater *et al.* 2016). When there is very severe drought, the formation of resting cells could also be a suitable mechanism for survival (McQuoid and Hobson 1996; Souffreau *et al.* 2013).

Resilience is the ability of organisms to reorganise pre-existing steady states (Acuña *et al.* 2015; Barthès *et al.* 2015). In general, changes in diatom community composition during a succession can be summarised as follows (McCormick and Jan Stevenson 1991): (1) facilitation (pioneer species dominate cobbles and produce autogenic habitat changes that favour secondary colonisers); (2) inhibition (the initial dominance of early colonisers inhibits late successional species colonisation and growth); (3) passive tolerance (interaction between early and secondary colonisers); and (4) active tolerance (increasing abundance of late successional species reduces the growth rate of early colonisers due to decreased availability of resources). Biofilm recovery after droughts strongly depends on several factors, such as predrought flow velocity, the speed of water retreat and the presence of refuges during the dry phase. In addition, river habitat integrity seems to be a key feature that affects the recovery of benthic communities (Calapez *et al.* 2014). However, one of the most important factors affecting biofilm recovery is the composition of algal assemblages during the predrought phase (Robson *et al.* 2008; Chester and Robson 2014). In this context, we should consider that Mediterranean streams are characterised by peculiar heterogeneous habitats that host unique and diverse biological assemblages. It has been observed previously that the stream identity in these

hydro-ecoregions strongly affects the composition of diatom assemblages, which can be very different even in streams that are close together (Falasco *et al.* 2016). This also highlights the distinctiveness of the Mediterranean diatom communities, which are often characterised by the presence of rare or endangered taxa (Novais *et al.* 2014; Falasco *et al.* 2016). Starting from these considerations, we addressed the following question in this study: do endangered species that colonise Mediterranean streams have the same resilience capacity as widespread species? It has been observed previously that diatoms are probably the most sensitive component of the autotrophic biofilm, because groups such as cyanobacteria and green algae cope better with droughts (Piano *et al.* 2016). Moreover, diatom species classified as threatened (according to Lange-Bertalot and Steindorf 1996) were shown to be highly sensitive to drought because their percentages decreased significantly with drought progression (Falasco *et al.* 2016). Indeed, Falasco *et al.* (2016) highlighted that the percentage of endangered species in a community should be considered as an important metric for evaluating hydrological disturbances. Despite this, to our knowledge no studies have investigated the resilience capacity of endangered species during drought, or their role in the recolonisation process.

Few studies have been performed with the aim of exploring recolonisation patterns in intermittent streams, especially in the Mediterranean hydro-ecoregion (Artigas *et al.* 2012; Calapez *et al.* 2014), despite the fact that improving our knowledge of diatom resilience and resistance after droughts is very important in a global climate change scenario. In recent years, several factors have exacerbated the extent of droughts in terms of frequency, duration and affected areas (Dudgeon *et al.* 2006; Fenoglio *et al.* 2010; Garcia *et al.* 2017). Moreover, hydrological models show a general decreasing trend of stream mean annual discharges, with an expected reduction of up to 30% during the 21st century (Schewe *et al.* 2014 cited in Garcia *et al.* 2017). Headwater stretches, characterised by perennial flow during the year, cannot be excluded from this forecast. Indeed, a significant negative decrease in stream discharge of perennial streams and springs has already been observed in Spain (Lorenzo-Lacruz *et al.* 2012; Martínez-Fernández *et al.* 2013). According to Deitch *et al.* (2017), the Ligurian region (north-west Italy) is classified as moderately dry in terms of annual precipitation (700–1300 mm), and shows weak seasonality (i.e. 12–20% of the annual precipitation falls during the summer months). However, analysing summer precipitation patterns in Liguria indicates a downward trend, suggesting that this region is becoming drier (Deitch *et al.* 2017). Indeed, the stretches affected by droughts are increasingly extending and, in recent years, flow has retreated, even reaching mountain sites (F.Bona and E. Falasco, pers. obs.).

The main aim of the present study was to analyse colonisation patterns of diatom communities that differ in terms of taxonomic composition and percentage of endangered taxa. To address this aim, we selected three streams based on results obtained in a previous study conducted in spring 2014 (Falasco *et al.* 2016); sampling sites were comparable in terms of water quality, but different in terms of surrounding land use. In each stream, we chose a sampling site with permanent flow throughout the whole year (hereinafter referred to as the 'experimental section') in which we performed two experimental treatments. The first treatment analysed the post-drought recolonisation process that is driven only by drift and immigration rates (hereinafter referred to as 'bare substrates [BS] treatment'). We hypothesised that differences in the starting community composition would lead to different colonisation patterns; moreover, communities with higher percentages of threatened species would be less resilient than those composed of widespread taxa. The second experimental treatment explored the possible contribution of potential resistance mechanisms within diatom communities (hereinafter referred to as 'transplanted substrates [TS] treatment'). To address this aim, cobbles were transplanted from a downstream section regularly affected by summer droughts and placed in the experimental section. We hypothesised that resistant forms should enhance the recovery of diatom communities, with significant differences in the colonisation patterns between BS and TS treatments.

Owing to the high and unpredictable hydrological variability of the stretches affected by droughts, we decided to perform the experiment in a section characterised by permanent flow during the whole year. The experimental sections in the study are the last sections of the streams characterised by permanent flow, which serve as a source of diatoms during the recolonisation process that takes place in the downstream sections (after water resumption). On the one hand, BS treatment simulates recolonisation on cobbles generally unaffected by drought, where diatom communities do not develop resistance mechanisms, whereas on the other hand TS treatment mimics recolonisation on substrates regularly affected by drought, where diatom communities probably develop resistance mechanisms.

Overall, the purpose of the present study was to mimic future possible scenarios in areas currently experiencing global climatic changes due to the rapid and marked extension of stretches affected by summer droughts. In this context, the results of this study will shed light on the resilience capacity of diatom species colonising Mediterranean streams, with a special focus on the role of endangered taxa on the recovery of communities.

Materials and methods

Sampling sites

Three Mediterranean streams (Arrestra, Quiliano and Sansobbia in Liguria, north-west Italy) characterised by summer flow intermittency in their downstream stretches were sampled in the present study. Streams were chosen using a selection process based on chemical data (historical series provided by the Environmental Agency of Liguria, ARPAL, see http://www.ambienteinliguria.it, accessed 13 July 2015) and the percentage of natural land use derived from an analysis of the Corine Land Cover (http://www.cartografia.regione.liguria.it/, accessed 16 July 2015).

We focused on streams located close to each other (within 20 km) with good water quality in order to avoid possible confounding effects due to differences in bed width, water chemistry, geology or climate. These three streams differed only in terms of surrounding natural land use, which was calculated within a 1-km buffer around each sampling site, and local human alterations (Fig. 1; see Table S1, available as Supplementary material to this paper). Arrestra can be considered as the most pristine stream, located in natural surroundings with almost no local human alterations (only 4% occupied by

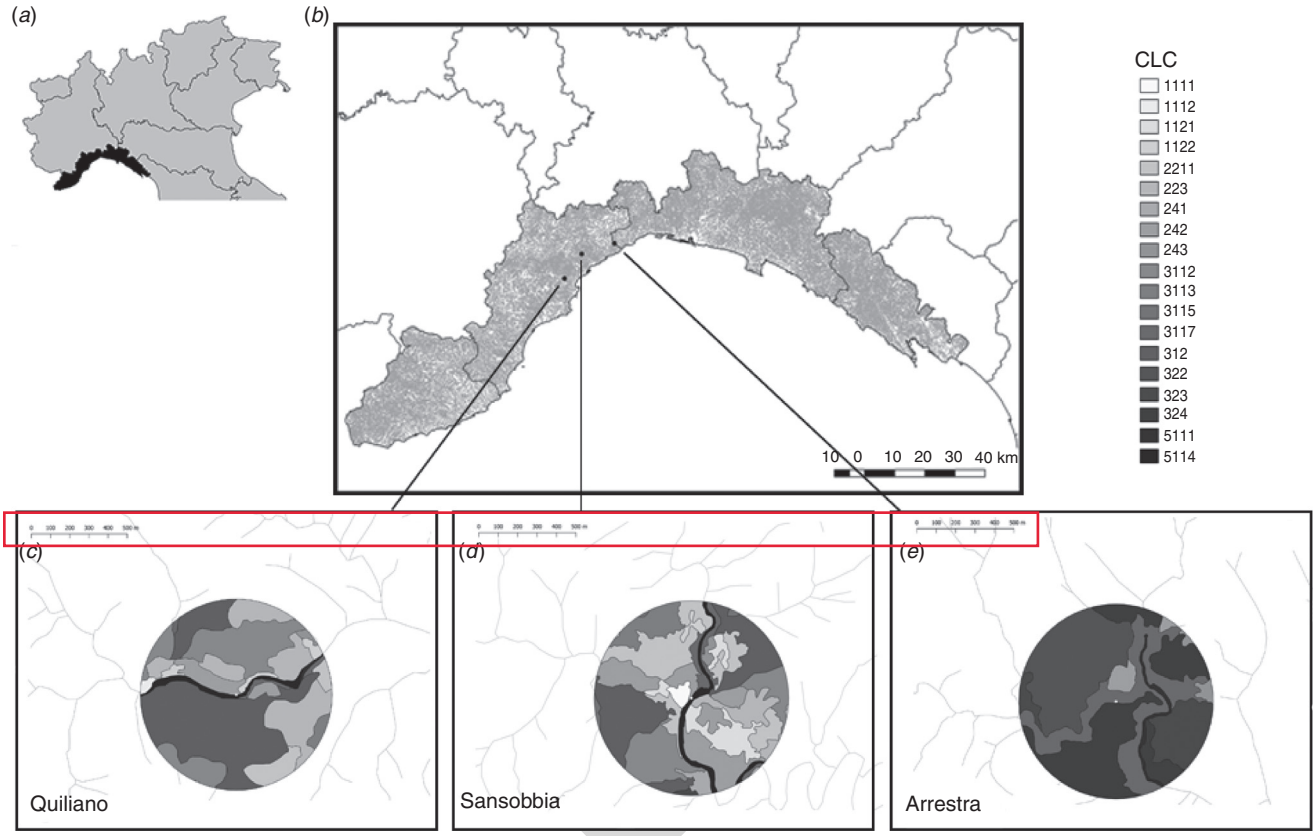

Fig. 1. (*a*, *b*) Sampling site locations and land use within a 1-km buffer around (*c*) Quiliano, (*d*) Sansobbia and (*e*) Arrestra stations. The different shades of grey used in the buffer are shown in the legend column and represent the Corine Land Cover (CLC) codes (for further details, see Table S1).

crops). The total percentage of natural land use was 94%, which consisted primarily of transitional woodland shrub, moors and heathlands. The riverbanks and streambed were both natural, and had large rocks and cobbles as the main mineral substrates. Quiliano represented a stream with an intermediate condition. The land use on the right riverbank consisted primarily of a continuous coniferous forest, with the exception of a small area with complex cultivation patterns. Human effects were limited to the left riverbank, on which small villages were located (2.6% of the buffer area), together with local agricultural activities, in particular olive groves (21% of the buffer area). The streambed was natural, with cobbles and gravel as the main substrates. The sampling stretch in Sansobbia was surrounded by natural land use in 44.2% of the buffer area. The stream was characterised by seminatural riverbanks and a natural riverbed, with cobbles and rocks as the main substrates. The sampling station was located just downstream of the village Ellera, whose urban fabric represented 10.1% of the buffer area. The main local agricultural activities (in total 42.2% of the buffer area) were complex cultivation patterns, vineyards and olive groves. Local human effects can still be considered limited in this stretch.

Experimental design

In each of the three streams, we chose one stretch characterised by permanent flow during the whole year. Experiments were performed with weekly samplings from October (t_0, experiment set-up) to December 2015 (t_{42}, i.e. 6 weeks after the experiment started). A final sampling campaign was conducted in January 2016 (t_{91}, 13 weeks after the experiment started). In all, there were seven sampling campaigns and 189 diatom samples were collected. At each site, diatom communities were sampled from both control and treated cobbles. Control communities (hereinafter referred to as 'C') were collected on completely submerged streambed cobbles, whereas two different treatments were tested in order to study the colonisation patterns of diatom communities.

BS treatment

The aim of the BS treatment was to investigate colonisation on completely bare substrates. With this treatment, we wanted to analyse what happens when the post-drought recolonisation process is driven only by drift and immigration rates. To this end, in each of the three streams, 30 cobbles were chosen from riverbanks in completely dry areas ($n = 21 + 9$ extra cobbles kept as spare substrates in case of loss). To ensure the total absence of diatoms, the cobbles were scraped with a metal brush and, at the same time, washed with 90% ethanol. At t_0, these 30 treated cobbles per site were marked and placed on the streambed.

TS treatment

The TS treatment was performed to assess the recolonisation patterns of diatom communities on cobbles regularly affected by

summer droughts. In each of the three streams, the closest sampling site that was affected by drought during the preceding months was identified. At time t_0, 30 cobbles from each of these downstream sites ($n = 21 + 9$ extra cobbles kept as spare substrates in case of loss) were marked and transplanted in their respective experimental sections in order to analyse diatom recolonisation patterns. The aim of the TS treatment was to determine whether diatom communities constantly subjected to harsh droughts are somehow adapted to this kind of stress and recover in a shorter time than communities developing on bare substrates. For this part of the study, we hypothesised that, if the colonisation process was completed in a shorter time on transplanted compared with bare cobbles, then we could confirm a significant role of resistant diatom forms during the recovery process on cobbles regularly affected by drought.

In order to guarantee comparable environmental conditions, cobbles belonging to the control, BS and TS samples were interspersed on the streambed within a 10-m stretch.

Environmental variables

In each sampling stretch and during all sampling campaigns, we monitored water temperature, conductivity, dissolved oxygen and pH using a multiparametric probe (mod. Quanta,Hydrolab, Loveland, CO, USA). On each cobble selected for diatom analysis, water depth and flow velocity were measured using a current meter (Mod RHCM, Idromarambiente, Genova, Italy), positioned 0.05 m from the bottom.

Soluble reactive phosphorous (SRP) and nitrates ($N\text{-}NO_3$) were monitored at t_{21}, t_{35} and t_{42} in order to detect possible variations in nutrient content in the water column.

Diatom communities: sampling procedure and analyses

Benthic diatom communities were collected by brushing the biofilm from the upper surface of both control and experimental cobbles, following the same standard procedure (European Committee for Standardization 2003). During each sampling campaign and in each stream, three replicates of biofilm were collected from three different cobbles for each treatment (C, BS and TS). Samples were kept separately for subsequent community analysis. Thus, a total of nine samples was collected in each of the three streams ($9 \times 3 = 27$) during each sampling campaign ($27 \times 7 = 189$ diatom samples in total). After diatom collection, marks were removed from the cobbles and the cobbles were replaced on the riverbanks. Samples were preserved in ethanol and treated in the laboratory with hydrogen peroxide (30%) and HCl, following a standardised method (European Committee for Standardization 2003). Light microscope slides were mounted with Naphrax (Brunel Microscopes, Chippenham, UK). Diatom identification was based on several diatom floras and monographs, as well as recent taxonomic papers (Krammer and Lange-Bertalot 1986, 1988, 1991*a*, 1991*b*; Lange-Bertalot 1996, 2001, 2004; Krammer 1997*a*, 1997*b*, 2002, 2003; Reichardt 1999; Blanco *et al.* 2010; Hofmann *et al.* 2011; Bey and Ector 2013; Falasco *et al.* 2013; Ector *et al.* 2015). For each slide, we identified at least 200 valves. We chose to identify 200 and not 400 valves as suggested by the standard procedure for diatom enumeration because we did not want to assess the water quality of the sampling sites in this study. In the present study we performed a manipulative experiment under strictly controlled environmental conditions, and the three samples collected from the three different cobbles were, *de facto*, three replicates of the same treatment. We decided to follow this procedure primarily to satisfy the requirements of the principal response curve (PRC) analysis (for which at least three replicates for each treatment are needed to test for statistical significance). Diatom communities were analysed in terms of biodiversity, taxonomic composition and ecological guilds (Rimet and Bouchez 2012). In addition, the presence and relative abundance of endangered species (hereinafter RL_species) were evaluated based on the Red List drawn up by Lange-Bertalot and Steindorf (1996).

Statistical analyses

To explore potential effects of environmental parameters on diatom communities, we performed canonical correspondence analysis (CCA) using flow velocity, water depth, conductivity, pH, dissolved oxygen and temperature in the environmental matrix.

Differences in terms of RL_species abundance between the control communities of the three streams were investigated using analysis of variance (ANOVA). Given the high diversity observed between the three streams in terms of community composition and percentage of RL_species, subsequent analyses were performed by keeping the samples obtained from the three different streams completely separate. Moreover, the biological dataset coming from each stream was reduced, maintaining only the most frequent and abundant diatom taxa (e.g. recorded in at least 50% of the samples and representing at least 2% abundance within the communities).

Changes in the relative abundances of diatom species during the entire experiment were analysed using the PRC (van den Brink *et al.* 2009). Significance was tested by means of a Monte Carlo test with 999 permutations.

Finally, a two-way ANOVA was performed for each stream in order to verify the role of treatment (C *v.* BS) and time on the abundance of RL_species.

All analyses were performed using R software (R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org, accessed 15 June 2016) and two-sided $P < 0.05$ was considered as the threshold for statistical significance. CCA and PRC were performed using the package vegan (ver. 2.3–5, J. F. Oksanen, G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner, Oulu, Finland, see https://CRAN.R-project.org/package=vegan).

Results

Environmental variables

The three streams were comparable in terms of physical and chemical features during the entire course of the experiment (Table 1). Water flow velocity was slightly different from site to site, reflecting a more lotic character in Sansobbia than in Arrestra. Depth was comparable between stretches: water was deeper during the first weeks of the experiment, being shallower between t_{21} and t_{35} in all streams and deeper again at the end of the study. Conductivity was slightly (but not significantly)

Table 1. Physical and chemical parameters of the three streams during the study period

Data are given as the mean ± s.e.m for flow velocity and water depth. Experiments were performed with weekly sampling from October (t_0, experiment set-up) to December 2015 (t_{42}, i.e. 6 weeks after the experiment started), with a final sampling in January 2016 (t_{91}, 13 weeks after the experiment started). DO, dissolved oxygen; SRP, soluble reactive phosphorous; N-NO_3, nitrates; –, not detected, BDL, below detection limit (values <0.001 mg L^{-1})

Stream and sampling day	Flow velocity ($m\,s^{-1}$)	Water depth (cm)	Temperature (°C)	Conductivity ($\mu S\,cm^{-1}$)	DO ($mg\,L^{-1}$)	DO (%)	pH	SRP ($mg\,L^{-1}$)	N-NO_3 ($mg\,L^{-1}$)	Streambed width (m)
Arrestra										
t_7	0.06 ± 0.06	19.7 ± 4.4	11.4	180	9.66	88.3	7.45	–	–	7.0
t_{14}	0.02 ± 0.03	12.4 ± 3.6	12.6	186	n.d.	n.d.	7.45	–	–	5.5
t_{21}	0.00 ± 0.00	11.0 ± 3.3	12.9	221	7.68	72.8	7.31	0.021	0.78	7.0
t_{28}	0.03 ± 0.02	15.0 ± 3.3	11.8	232	8.93	82.1	7.24	–	–	4.8
t_{35}	0.00 ± 0.00	13.2 ± 3.4	11.1	236	9.60	87.1	7.23	BDL	0.75	4.6
t_{42}	0.21 ± 0.10	25.8 ± 1.4	9.88	150	10.10	89.9	7.41	BDL	1.10	6.3
t_{91}	0.01 ± 0.02	17.7 ± 2.7	5.00	168	11.10	86.8	6.80	–	–	6.0
Quiliano										
t_7	0.12 ± 0.13	15.0 ± 3.5	12.9	173	9.59	90.9	7.59	–	–	4.0
t_{14}	0.12 ± 0.06	15.6 ± 2.7	14.0	179	9.75	94.5	7.52	–	–	4.0
t_{21}	0.15 ± 0.09	11.9 ± 2.9	14.1	180	10.30	100	7.75	0.094	0.65	4.2
t_{28}	0.16 ± 0.07	14.6 ± 5.2	10.5	182	9.98	89.3	7.57	–	–	4.0
t_{35}	0.14 ± 0.09	13.8 ± 3.2	11.2	178	9.95	90.0	7.56	BDL	0.69	3.9
t_{42}	0.12 ± 0.01	13.6 ± 3.3	10.4	179	9.83	88.0	7.41	BDL	1.23	4.1
t_{91}	0.10 ± 0.08	16.1 ± 2.4	8.03	168	11.20	94.6	7.18	–	–	4.4
Sansobbia										
t_7	0.39 ± 0.19	15.4 ± 5.8	12.1	267	9.04	82.1	8.19	–	–	4.2
t_{14}	0.36 ± 0.19	16.2 ± 4.9	13.4	294	11.20	107.0	8.15	–	–	4.2
t_{21}	0.31 ± 0.18	13.2 ± 5.2	14.0	321	11.90	115.0	7.79	0.008	1.48	4.3
t_{28}	0.36 ± 0.20	13.2 ± 5.9	9.00	322	12.20	105.0	8.25	–	–	4.3
t_{35}	0.39 ± 0.24	11.2 ± 2.9	9.84	321	8.29	73.8	8.4	0.015	1.37	4.0
t_{42}	0.44 ± 0.20	18.3 ± 3.7	8.87	234	9.02	78.2	8.07	0.012	1.49	4.6
t_{91}	0.30 ± 0.19	15.9 ± 4.2	5.89	272	19.10	151.0	7.67	–	–	4.6

higher in Sansobbia than in the other two streams, and pH was neutral in both Quiliano and Arrestra and slightly alkaline in Sansobbia. Wet streambed width was almost constant during the entire experiment in Sansobbia and Quiliano, but varied more in Arrestra. Nutrient concentrations confirmed the good ecological status of the three streams: SRP levels fell into the highest quality class in all stretches and in all surveys; values of nitrates corresponded to the second highest class (Italian Legislative Decree 152/2006 'Norms concerning the Environment').

Diatom community composition

The first three axes of the CCA explained 82% of the total variance in the environmental variables across all streams and at all time points (Fig. 2; Table 2). The first axis alone accounted for 55% of the total variance and represented a slight gradient of eutrophication, being negatively correlated with conductivity, pH and flow velocity. The second axis (accounting for 15% of total variance) was negatively correlated with temperature, whereas the third axis (accounting 12% of total variance) was negatively correlated with dissolved oxygen and saturation. Visual inspection of the ordination shows a clear difference in the three streams, with higher values of conductivity, pH, dissolved oxygen and flow velocity in Sansobbia, higher water depth in Quiliano and higher temperature in Arrestra. The clear distinction of the three streams in terms of diatom species composition underlies the key role of stream identity. According to temporal variability, there was no clear trend evident throughout the sampling season. Considering this, any changes observed in the community composition from t_7 to t_{91} could be possibly ascribed to the different treatments.

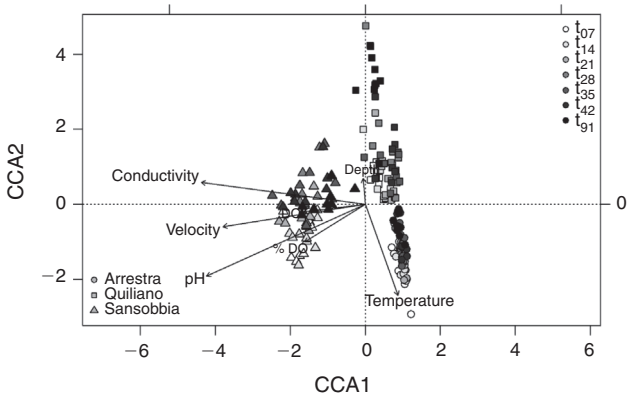

Fig. 2. Canonical correspondence analysis (CCA) diagram showing environmental parameters and sampling sites in the ordination space. Shades of grey represent the sampling sessions during the experiment, with each stream allocated a different shape. DO, dissolved oxygen.

Table 2. Canonical correspondence analysis (CCA): axes summary statistics
DO, dissolved oxygen

	CCA1	CCA2	CCA3
Eigenvalue	0.421	0.1172	0.08719
Proportion explained	0.5554	0.1546	0.11503
Cumulative proportion	0.5554	0.71	0.82505
Velocity	−0.7265	−0.1575	0.17245
Depth	−0.01059	0.1814	−0.1532
Temperature	0.16703	−0.63992	0.31198
Conductivity	−0.83699	0.15428	−0.05575
DO	−0.31619	−0.05432	−0.68916
%DO	−0.30957	−0.26572	−0.60524
pH	−0.81042	−0.50469	0.15826

The complete list of diatom species detected during the entire experiment is presented in Table S2, whereas results concerning the number of genera and species identified, Shannon diversity index and evenness are given in Table 3. Overall, 91 species were detected in Arrestra, the most pristine stream. In terms of ecological guilds, approximately 40% of species were classified as low profile (i.e. prostrate, adnate and erect diatoms resistant to physical disturbances but sensitive to nutrient enrichment), 34% were classified as high profile (i.e. large and colony-forming diatoms that are not resistant to physical disturbance but are stimulated by nutrient enrichment), 22% were classified as motile (i.e. fast-moving species adapted to both physical disturbance and nutrient enrichment) and approximately 4% were classified as planktonic (i.e. adapted to lentic environments). The most frequent and abundant low-profile species belonged to the genera *Achnanthidium* (*A. affine*, *Achnanthidium* sp., *A. gracillimum, A. lineare* and *A. minutissimum*) and *Encyonopsis* (*E. minuta* and *E. subminuta*. Several high-profile taxa (*Diatoma ehrenbergii*, *Encyonema ventricosum*, *Fragilaria rumpens* and *Ulnaria biceps*) were detected in considerable proportions, whereas only one motile species was included among the most frequent and abundant taxa (*Nitzschia fonticola*). In total, 94 species were detected in the Quiliano stream, of which 35% were classified as low profile, 22% as high profile, and 43% as motile. Among the low-profile species, the most frequent and abundant species were *Achnanthidium* sp., *A. minutissimum*, *Achnanthidium pyrenaicum*, *Amphora pediculus*, *Cocconeis lineata*, *Planothidium lanceolatum* and *Reimeria sinuata.* Among the high-profile species, we frequently detected *E. ventricosum* and *Gomphonema parvulum*, whereas the motile species were primarily *Navicula gregaria*, *Nitzschia dissipata* and *Nitzschia fonticola.* In all, 104 species were detected in Sansobbia, of which 33% were classified as low profile, 22% as high profile, 42% as motile and 3% as planktonic. Among the low-profile species, the most frequent and abundant taxa belonged to the genera *Achnanthidium* (*A. eutrophilum*, *A. minutissimum* and *A. pyrenaicum*), *Amphora* (*A. pediculus*), *Cocconeis* (*C. euglypta, C. pediculus* and *C. placentula* teratological form) and *Reimeria* (*R. sinuata*). The most frequent and abundant motile taxa were *Fistulifera saprophila*, *Geissleria decussis*, *Mayamaea permitis*, *Navicula antonii*, *Navicula cryptotenella*, *N. gregaria*, *Navicula reichardtiana*, *N. dissipata*, *N. fonticola*, *Nitzschia inconspicua* and *Sellaphora nigri*. Even though sites were specifically selected because they had low human disturbance, these species are known for their low sensitivity to anthropogenic pressures. According to the CCA results, these taxa (in particular *Craticula subminuscula*, *F. saprophila*, *M. permitis* and *Nitzschia tabellaria*) were positively correlated with conductivity, flow velocity and pH.

The results of the one-way ANOVA showed that the relative abundance of RL_species was highly stream dependent ($F_{2,60} = 100$; $P < 0.001$) and these differences were significant between all three streams (Tukey *post hoc* test, $P < 0.001$). Arrestra showed the highest RL_species abundance during the entire survey (median value 17.0%). The most frequently detected RL_species were: *A. gracillimum* (found in 100% of samples analysed, with a mean relative abundance of 12%), *U. biceps* (detected in ~70% of samples, with a mean relative abundance of 1.4%) and *A. lineare* (found in half the samples, with a mean relative abundance of 1.1%). In Quiliano, median RL_species abundance during the entire survey was 3.8%. The most frequent RL_species were *A. lineare* (observed in ~43% of samples, with a mean relative abundance of 1.4%), followed by *Nitzschia oligotraphenta* (in ~40% of samples, with a mean relative abundance of 1%) and *A. gracillimum* (detected in ~37% of samples, with a mean relative abundance of ~0.5%). Very few endangered species were detected in Sansobbia (median value 0.5%); these species were *A. gracillimum* and *A. lineare* (found in 16 and 14% of samples analysed respectively, with a mean relative abundance of 0.1 and 0.2% respectively) and *N. oligotraphenta* (detected in ~13% of samples, but with a very low relative abundance (mean 0.1%)).

Colonisation pattern and recovery

For each of the streams investigated, a PRC analysis was performed to explore differences in the pattern of diatom colonisation between the three groups (C, BS, TS) over time (Fig. 3). This analysis allowed graphical visualisation of the recovery (if present) of diatom communities on the treated compared with control cobbles; moreover, the comparison between BS and TS patterns of colonisation gave us the possibility to highlight the possible role (if present) of resistant forms in the recolonisation process. Comparing the BS and TS results with C data showed highly significant differences for all three streams, with variable patterns.

In the Arrestra stream, despite a very simple and homogeneous species composition, the diatom communities were different on the treated compared with control cobbles throughout the entire experiment (Fig. 3). These differences were still evident at t_{91}. In terms of diatom species composition, this was due primarily to the relative abundance of just a few species: *A. gracillimum* and *E. minuta* were the key species on the control cobbles. If we focus on the conservation status of diatom species, we can highlight that endangered species, such as *A. gracillimum* and *A. lineare*, were the most affected by the treatments, and their absence from BS and TS cobbles strongly contributed to differences with control samples. Treated communities were generally dominated by *A. minutissimum*, a cosmopolitan species with a low conservation value

Table 3. Total number of genera and species identified, Shannon diversity index and evenness recorded during the experiment in the three treatment groups

Data are given as the mean ± s.d., calculated from the three replicates for each treatment. Experiments were performed with weekly sampling from October (t_0, experiment set-up) to December 2015 (t_{42}, i.e. 6 weeks after the experiment started), with a final sampling in January 2016 (t_{91}, 13 weeks after the experiment started). C, control; BS, bare substrates; TS, transplanted substrates

Stream and sampling day	Treatment	Number of genera	Number of species	Shannon diversity index	Evenness
Arrestra					
t_7	C	9.3 ± 2.1	17.7 ± 2.5	2.3 ± 0.2	0.6 ± 0.0
	BS	10.7 ± 3.2	21.0 ± 6.1	2.5 ± 0.6	0.6 ± 0.1
	TS	9.0 ± 1.0	17.3 ± 1.2	2.2 ± 0.2	0.5 ± 0.1
t_{14}	C	11.3 ± 2.3	19.0 ± 3.6	2.3 ± 0.4	0.5 ± 0.1
	BS	9.7 ± 1.5	17.0 ± 3.5	2.2 ± 0.2	0.5 ± 0.0
	TS	10.3 ± 2.1	19.3 ± 3.1	2.1 ± 0.2	0.5 ± 0.0
t_{21}	C	8.3 ± 2.3	14.7 ± 3.1	2.0 ± 0.3	0.5 ± 0.1
	BS	12.7 ± 0.6	23.3 ± 3.2	2.5 ± 0.4	0.6 ± 0.1
	TS	9.0 ± 1.0	16.3 ± 2.1	1.6 ± 0.3	0.4 ± 0.1
t_{28}	C	9.7 ± 1.2	18.0 ± 4.4	2.0 ± 0.2	0.5 ± 0.0
	BS	9.3 ± 0.6	15.7 ± 1.5	1.6 ± 0.2	0.4 ± 0.0
	TS	7.3 ± 1.5	13.3 ± 3.2	1.3 ± 0.2	0.3 ± 0.0
t_{35}	C	9.7 ± 3.5	16.3 ± 5.7	2.0 ± 0.7	0.5 ± 0.1
	BS	10.7 ± 2.1	18.7 ± 3.8	2.3 ± 0.2	0.5 ± 0.1
	TS	8.7 ± 3.5	12.7 ± 4.2	1.5 ± 0.6	0.4 ± 0.1
t_{42}	C	11.7 ± 2.3	19.3 ± 3.1	2.6 ± 0.2	0.6 ± 0.1
	BS	11.0 ± 1.7	19.3 ± 3.1	2.6 ± 0.5	0.6 ± 0.1
	TS	10.0 ± 0.0	16.7 ± 1.5	2.1 ± 0.4	0.5 ± 0.1
t_{91}	C	10.7 ± 1.5	20.3 ± 2.5	2.7 ± 0.2	0.6 ± 0.0
	BS	9.3 ± 0.6	15.7 ± 1.5	1.8 ± 0.2	0.5 ± 0.0
	TS	9.7 ± 1.5	16.7 ± 3.2	2.2 ± 0.3	0.5 ± 0.0
Quiliano					
t_7	C	12.7 ± 1.5	24.7 ± 4.0	3.1 ± 0.3	0.7 ± 0.0
	BS	16.0 ± 1.0	33.7 ± 0.6	3.8 ± 0.2	0.7 ± 0.0
	TS	14.7 ± 1.2	26.7 ± 0.6	3.4 ± 0.1	0.7 ± 0.0
t_{14}	C	12.3 ± 1.2	19.7 ± 1.2	2.6 ± 0.3	0.6 ± 0.1
	BS	10.0 ± 1.7	17.0 ± 2.0	2.1 ± 0.1	0.5 ± 0.0
	TS	12.3 ± 2.1	19.7 ± 4.0	2.4 ± 0.9	0.6 ± 0.2
t_{21}	C	10.3 ± 1.2	17.0 ± 1.7	2.0 ± 0.1	0.5 ± 0.0
	BS	8.0 ± 1.0	11.3 ± 3.5	1.1 ± 0.4	0.3 ± 0.1
	TS	9.3 ± 1.5	14.7 ± 4.0	1.4 ± 0.5	0.4 ± 0.1
t_{28}	C	10.3 ± 2.5	15.0 ± 2.6	1.9 ± 0.4	0.5 ± 0.1
	BS	6.3 ± 0.6	8.0 ± 1.7	0.9 ± 0.2	0.3 ± 0.1
	TS	9.0 ± 1.0	13.0 ± 1.0	1.7 ± 0.1	0.4 ± 0.0
t_{35}	C	11.7 ± 2.9	18.0 ± 4.6	2.4 ± 0.6	0.6 ± 0.1
	BS	6.0 ± 2.0	10.3 ± 2.1	1.1 ± 0.2	0.3 ± 0.0
	TS	3.7 ± 0.6	8.3 ± 1.2	1.1 ± 0.2	0.4 ± 0.1
t_{42}	C	11.3 ± 3.5	18.3 ± 6.5	2.4 ± 0.9	0.6 ± 0.2
	BS	6.0 ± 3.0	10.7 ± 3.8	1.2 ± 0.3	0.4 ± 0.0
	TS	6.0 ± 0.0	11.7 ± 1.2	1.4 ± 0.2	0.4 ± 0.1

(*Continued*)

Table 3. (Continued)

Stream and sampling day	Treatment	Number of genera	Number of species	Shannon diversity index	Evenness
t_{91}	C	11.7 ± 2.1	22.0 ± 3.6	3.3 ± 0.1	0.7 ± 0.0
	BS	10.0 ± 1.7	17.7 ± 4.0	2.6 ± 0.4	0.6 ± 0.1
	TS	11.0 ± 2.0	19.7 ± 1.2	3.0 ± 0.2	0.7 ± 0.1
Sansobbia					
t_7	C	10.7 ± 2.1	21.7 ± 6.1	2.9 ± 1.0	0.7 ± 0.2
	BS	17.0 ± 2.6	30.7 ± 1.5	3.8 ± 0.2	0.8 ± 0.0
	TS	17.0 ± 2.0	33.3 ± 4.2	4.2 ± 0.1	0.8 ± 0.0
t_{14}	C	9.3 ± 1.2	16.7 ± 3.2	2.9 ± 0.3	0.7 ± 0.0
	BS	15.0 ± 1.7	23.7 ± 2.5	3.3 ± 0.2	0.7 ± 0.0
	TS	15.7 ± 0.6	27.0 ± 1.0	3.4 ± 0.1	0.7 ± 0.0
t_{21}	C	12.7 ± 0.6	26.3 ± 3.1	3.6 ± 0.2	0.8 ± 0.0
	BS	11.7 ± 4.7	22.3 ± 9.3	2.9 ± 1.2	0.6 ± 0.2
	TS	13.0 ± 2.6	24.0 ± 3.5	3.2 ± 0.3	0.7 ± 0.0
t_{28}	C	12.0 ± 2.0	27.0 ± 3.6	3.7 ± 0.3	0.8 ± 0.0
	BS	13.3 ± 1.2	22.7 ± 3.8	3.3 ± 0.4	0.7 ± 0.1
	TS	12.0 ± 1.0	20.3 ± 3.2	3.2 ± 0.3	0.7 ± 0.0
t_{35}	C	11.0 ± 2.6	18.3 ± 5.0	3.2 ± 0.4	0.8 ± 0.0
	BS	13.3 ± 4.0	20.3 ± 6.5	3.3 ± 0.3	0.8 ± 0.0
	TS	10.3 ± 2.3	17.7 ± 3.2	2.9 ± 0.3	0.7 ± 0.1
t_{42}	C	12.3 ± 1.2	22.3 ± 3.8	3.3 ± 0.2	0.7 ± 0.0
	BS	10.7 ± 2.9	16.3 ± 4.2	2.8 ± 0.2	0.7 ± 0.0
	TS	12.0 ± 1.0	19.7 ± 3.2	3.2 ± 0.2	0.7 ± 0.0
t_{91}	C	13.7 ± 0.6	24.7 ± 0.6	3.3 ± 0.3	0.7 ± 0.1
	BS	13.7 ± 0.6	23.3 ± 2.5	3.2 ± 0.2	0.7 ± 0.0
	TS	13.3 ± 2.1	22.3 ± 3.1	3.2 ± 0.1	0.7 ± 0.1

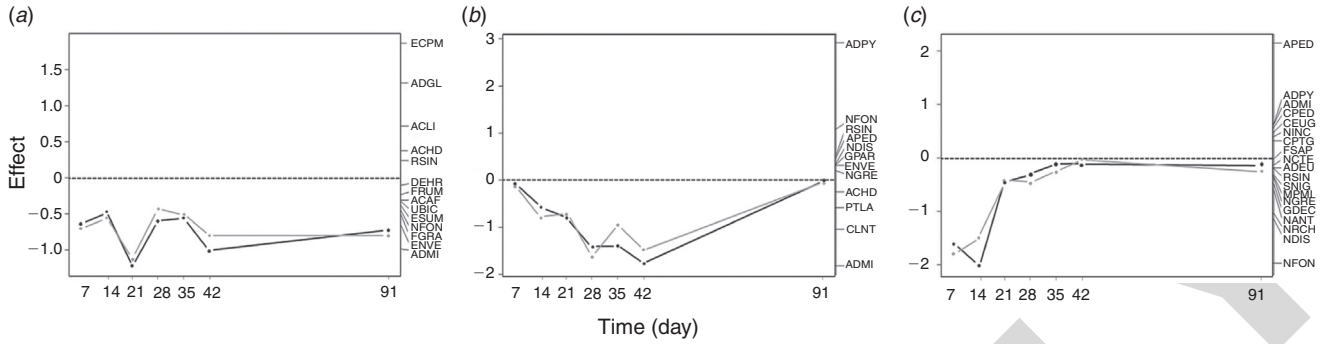

Fig. 3. Principal response curves diagram of the diatom communities exposed to different kinds of treatment in the (*a*) Arrestra, (*b*) Quiliano and (*c*) Sansobbia streams. Dashed lines represent the community composition of the control (C) from Day 7 (t_7) to Day 91 (t_{91}). Grey (bare substrates (BS) treatment) and black (transplanted substrates (TS) treatment) lines represent the patterns of treated diatom community composition compared with controls. Species weights (on the right) indicate the relative contribution of individual species to the community response. For definitions of codes on the right-hand side of each graph, see Table S2.

(Falasco *et al.* 2013). Compared with Arrestra, the initial diatom communities in the Quiliano stream were more diverse in terms of both ecological guilds and species composition. This probably contributed to the recovery of the treated communities, which were very similar to the control at t_{91}. It seems that most of the species found in the control samples started to colonise treated cobbles after just 1 week (t_7). Subsequently, it is possible that several factors, such as inter- and intraspecific competition dynamics, contributed to shaping the treated communities. The main difference between the control and treated communities was again due to just a few species: *A. pyrenaicum* primarily dominated in C, whereas *A. minutissimum* was typical of the treated assemblages. Diatom communities in the Sansobbia stream were the most efficient in terms of recovery (Fig. 3). Already at t_{28}, no significant differences between the treated and control communities were observed, and this similarity was maintained throughout the remaining experimental period. Diatom communities in the Sansobbia stream were the most diverse in terms of species composition. The differences between the control and treated samples at t_7 were due primarily to motile taxa belonging to the genera *Nitzschia* (*N. fonticola* and *N. dissipata*) and *Navicula* (*N. reichardtiana*) on BS and TS. Conversely, control assemblages were dominated by *A. pediculus*, *A. minutissimum* and *C. euglypta*.

Results of PRC analysis showed an almost identical pattern between communities colonising BS or TS throughout the entire experiment. Based on this finding, we can deduce that TS did not offer a more suitable substrate for diatom colonisation than BS. Because we could assume that the communities developing on BS and TS were comparable, the following analyses were performed comparing only C and BS treatment.

In the Arrestra stream, two-way ANOVA (Table 4) detected significant differences in the abundance of endangered taxa during the entire experiment, with percentages of RL_species consistently much higher in C than BS (see also Fig. 4). Concerning time, the test highlighted significantly lower values of RL_species at t_{91} than at t_7 and t_{21} (Tukey *post hoc* test, $P < 0.045$). Again, in Quiliano, the percentage of RL_species was consistently much higher in C than BS. However, in this case we noticed a significant progressive decreasing trend from t_7–t_{14} towards t_{21}–t_{28} and then from t_{21}–t_{28} towards t_{35}, but there was then a significant increase from t_{42} towards t_{91} (Tukey *post hoc* test, $P < 0.041$) in both C and BS. Differences in the percentage of RL_species between C and BS were not significant at t_{91}, when the abundance of endangered species was comparable to that at the beginning of the experiment. In Sansobbia, as expected, two-way ANOVA did not reveal any significant difference in terms of RL_species abundance between C and BS; indeed, in both cases, the percentages were very low.

Table 4. Results of the two-way analysis of variance (ANOVA) performed to detect significant differences in terms of abundance of endangered species between treatments and time points

	Treatment	Day
Arrestra	$F_{1,34} = 13.8$; $P < 0.001$	$F_{6,34} = 2.94$; $P = 0.020$
Quiliano	$F_{1,34} = 5.38$; $P = 0.026$	$F_{6,34} = 17.7$; $P < 0.001$
Sansobbia	$F_{1,34} = 0.568$; $P = 0.456$	$F_{6,34} = 1.27$; $P = 0.295$

Discussion

The present study highlighted significant differences in terms of diatom community recovery depending on the assemblage composition. Despite the geographical closeness of the three systems studied, stream identity had a strong effect on diatom community assemblages, in accordance with our previous results obtained in the same area (Falasco *et al.* 2016). In that study, differences in terms of composition were driven primarily by conductivity (with 400 µS cm^{-1} as the threshold level; Falasco *et al.* 2016). In the present study, conductivity was always lower than 350 µS cm^{-1}; despite this, the results of the CCA showed a strong effect of conductivity on diatom communities, together with pH and flow velocity.

CCA also provided information concerning the ecological preferences of some species. Among the most interesting results, we observed that *C. subminuscula*, *F. saprophila*, *M. permitis* and *N. tabellaria* were favoured by higher conductivity, pH and flow velocity. Moreover, *Fragilaria neointermedia*,

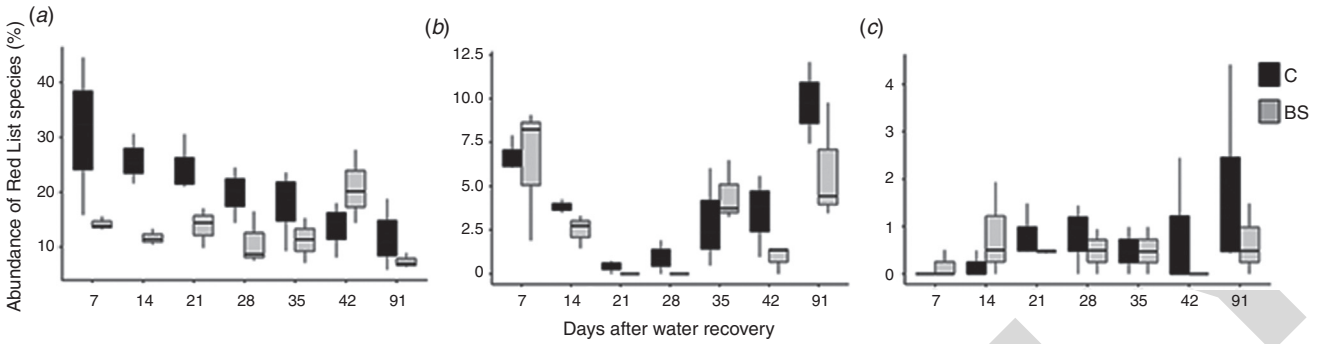

Fig. 4. Boxplots showing the percentage abundance of endangered species on the control cobbles (C) and bare substrates (BS) during the entire experiment in the (*a*) Arrestra, (*b*) Quiliano and (*c*) Sansobbia streams. The boxes show the interquartile range, with the median value indicated by the horizontal line; whiskers show the range.

Gomphonema italicum, *Nitzschia heufleriana*, *Staurosira binodis* and *Rhoicosphenia abbreviata* were more abundant in stretches characterised by high levels of dissolved oxygen.

In the present study, we chose to sample stretches characterised by comparable physical and chemical features, but with different levels of surrounding natural land use in order to assess whether diatom communities, notably different in terms of taxonomic composition, ecological guilds and percentage of endangered species, showed differences in recolonisation patterns. As already highlighted in previous studies, the Mediterranean streams selected in this study shelter many species considered as threatened to different degrees and, of the diatoms, these taxa must be considered as the most sensitive species to hydrological alterations (Falasco *et al.* 2016).

In general, we found that the early stage of diatom recolonisation took just 1 week, and many different species were already collected from the treated cobbles at t_7. This finding can be interpreted as an adaptive mechanism of communities living in Mediterranean intermittent streams, and is comparable with the results reported by Artigas *et al.* (2012). In that study, the authors observed a faster recolonisation on BS in a Mediterranean intermittent stream compared with a slow, gradual recovery in a Central European river, the Walzbach.

In particular, in Arrestra, the most abundant species belonged to the low-profile guild; these species were *A. minutissimum* and *A. gracillimum*. This latter species is included in the diatom Red List as 'endangered' (Lange-Bertalot and Steindorf 1996), and is considered a sensitive species typical of calcareous rivers with low organic matter and nutrient content (Ponader and Potapova 2007; Hofmann *et al.* 2011; Bey and Ector 2013). *A. gracillimum* is primarily associated with shallow standing or flowing waters with the main substrate being microlithal (Falasco *et al.* 2016). In the present study, *A. gracillimum* represented the key species that exhibited differences between control and treated communities, showing a consistent and significant decrease in treated communities. The same pattern was evident for *A. lineare*, but to a lesser degree. *A. lineare* is also classified as 'endangered' by Lange-Bertalot and Steindorf (1996) and is typical of oligotrophic streams with circumneutral-to-alkaline pH and low–moderate conductivity values (Van de Vijver *et al.* 2011). The first 2 weeks of the recolonisation on treated cobbles were dominated primarily by *A. minutissimum.* Indeed, this is generally recognised as a pioneer species, with high growth rates (Rimet and Bouchez 2012). *A. minutissimum* showed early colonisation on BS and TS and, after just 1 week, already represented more than 50% of the diatom communities. Other taxa also seemed to characterise these first stages of the colonisation process, but with significantly lower abundances. Species belonging to the genus *Fragilaria*, for example, were more abundant on treated than control cobbles, confirming the observations of Sabater *et al.* (2016). In our case, Arrestra diatom communities of the treated cobbles were still significantly different from control cobbles at t_{91}, with an important loss of endangered species. The two-way ANOVA highlighted a general decrease in endangered species from t_7 to t_{91} in control communities as well.

Diatom assemblages in Quiliano and Sansobbia were more heterogeneous in terms of both taxonomic and functional composition. The recolonisation in Quiliano seemed to be very rapid, and communities were completely able to recover at t_{91}. Indeed, at t_7 we already observed the presence of 'S' selected taxa (for further details, see Grime 1977; Morin *et al.* 2008), such as *N. gregaria*, *P. lanceolatum* and *G. parvulum*. These are considered slow colonisers with intermediate growth rates, and should arrive in a second phase of recolonisation. In Quiliano, at t_7, the percentage relative abundance of 'R' selected taxa on the BS cobbles were comparable to those seen on C cobbles, highlighting the end of the first colonisation stage. We can therefore hypothesise that, in Quiliano, the communities were able to recover in just 1 week. Despite this, at t_{14}, this trend inverted and the pioneer taxa again dominated the samples, leading to the considerable deviation seen in Fig. 3. We can hypothesise that possible interspecific competition favoured the presence of more tolerant taxa (e.g. 'S' selected) during the first days of the experiment, to the detriment of the most sensitive ones (e.g. species belonging to the genus *Achnanthidium*). The two-way ANOVA highlighted a general decrease in endangered species in December (t_{14}–t_{35}) in both C and BS groups; this was due primarily to the progressive reduction in the relative abundance of *N. oligotraphenta* and *Navicula splendicula* (both classified as 'declining'). Conversely, we did not detect a significant difference in the percentage of RL_species among C and BS groups during the final sampling campaign, with the percentage at this time being comparable to that recorded at t_7.

In this case, the trend was driven primarily by *A. lineare* (classified as 'endangered'). This species appeared in the stretch during winter, at the end of the experiment, and did not show preferences in terms of substrate, being able to colonise both C and BS to the same extent. In both Arrestra and Quiliano, we observed a decreasing and increasing trend respectively in RL_species during the experiment, as well as on C cobbles. The factors driving this trend are unclear. We first hypothesised a seasonal preference of some RL_species, but finally excluded this possibility after checking the single species trends in the two streams. For example, we observed a marked decrease in *A. gracillimum* abundance from October to January in Arrestra, but the inverse tendency in Quiliano, where the species doubled at the end of the experiment. At the same time, *A. lineare* increased from 0% at t_7 up to 7% at t_{91} in Quiliano, but decreased gradually in Arrestra during winter. From our results, we cannot exclude the role of interspecific competition or of stochastic events to explain the trends in RL_species. It is clear that further information on species autecology could provide important insights to interpret these results.

The recovery was even more efficient and fast in Sansobbia ($\sim t_{28}$–t_{35}). The communities on the C cobbles were highly heterogeneous, in terms of both taxonomic composition and ecological guild. A significant percentage of these communities comprised pioneer taxa, such as *A. minutissimum* and *A. pediculus*, which probably competed for space and resources on BS and TS. In this case, we did not observe a strong dominance of *A. minutissimum* on the treated cobbles, and this probably represented an advantage for late successional species, such as the motile taxa *N. dissipata*, *N. fonticola* and *N. reichardtiana*. These diatom assemblages, highly heterogeneous in terms of growth forms, probably produced thickened mats of cells and mucilage, which contributed to the entrapment of other algae and acted as a basal layer (Morin *et al.* 2008). At the same time, the upper layers probably limited the growth of the low-profile early colonisers due to the decrease in internal nutrient recycling (Jan Stevenson and Glover 1993). In this case, we did not detect significant percentages of RL_species, and this was probably why we observed such fast and total recovery.

The results on this study contrast, in part, with those reported by Calapez *et al.* (2014), who showed that communities of highly natural stretches recovered faster than assemblages of disturbed areas. In the present study, sites were selected among the least affected streams of the Ligurian region, and differences between the streams were due primarily to the percentage of natural land use. Despite this, local human effects characterising our stretches can be considered as very limited. The differences we found in the recovery patterns were probably only related to the initial composition of assemblages and abundance of endangered species.

The similarity of diatom composition in the BS and TS groups during the entire experiment (Fig. 2) confirmed the results obtained in previous studies. According to these studies, the main source of diatoms during the post-drought process is thought to be drift and migration from colonised areas, more than from dry biofilm (Chester and Robson 2014; Barthès *et al.* 2015). The present study did not aim to highlight the possible presence of resistant forms in the dry biofilm, only their possible positive role during the recolonisation process. However, contrary to our hypothesis, through PRC analysis we were able to show that, even if present, resistant forms did not enhance the recovery ability of the biofilm, because colonisation patterns between bare and transplanted substrates were almost identical.

Conclusion

The growing extent of the hydrological intermittency in Mediterranean areas is worrying, as are the consequences on benthic biological communities, especially those at the base of the food web. Droughts affect diatom primary production (Piano *et al.* 2016) and modify community composition with a significant loss of unusual endangered species (Falasco *et al.* 2016). Simple communities, such as those colonising the most pristine environments, which comprise a significant percentage of endangered species, are potentially the most affected by water absence. Based on our results, if subjected to extreme drought, these assemblages do not show complete recovery in terms of species composition, even 3 months after the water returns. Conversely, more heterogeneous communities composed of widespread taxa showed higher resilience and were able to completely recover after just a few weeks.

Management of aquatic resources in areas characterised by high interannual variability in the amount of water is very difficult but, at the same time, is very important because these systems can be important refuges for biodiversity (Ros *et al.* 2009). In this context, management plans that consider the possibility of facing long periods of dry are needed. Perennial headwater streams cannot be excluded from these plans, and further research on the effects of hydrological intermittency in these stretches is required.

Conflict of interest

The authors declare that they have no conflicts of interest.

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AQ1: What version of R?